SIGNAL PROCESSING APPARATUS AND METHOD

The present invention relates to a processing apparatus and method, and particularly though not exclusively to a normalisation apparatus and method.

It is well known to measure a signal which is emitted from a sample in response to excitation of that sample. Where this is done, it is common to monitor the excitation of the sample so that the experimental signal may be normalised according to a property of the excitation. Normalisation allows the experimental signal to be corrected to reduce the effect of fluctuations of the excitation. Normalisation is commonly used in fluorescent studies, and is of particular value when pulsed excitation of a fluorescent sample is used.

In one known normalisation method the intensity of fluorescence emitted by a sample is recorded together with the intensity of the excitation signal, and normalisation is carried out after the data has been stored, for example by dividing the fluorescence intensity by the excitation signal. This normalisation method is limited to the analysis of a fluorescent signal which has sufficient intensity that it may be detected as an analogue signal, and is not suitable for normalisation of fluorescence which is detected as a series of discrete photons. The method also suffers from the disadvantage that it does not take place in real-time.

In an alternative known normalisation method, an average intensity of a pulsed excitation is determined and is used to normalise a recorded detected fluorescence signal. However, this method does not take account of inter-pulse intensity variations. These variations are important because they may lead to non-linear fluorescence effects, for example two photon fluorescence.

It is an object of the present invention to provide a processing method and apparatus which overcomes at least some of the above disadvantages.

According to a first aspect of the invention there is provided a normalisation apparatus arranged to normalise a first signal with respect to a second related signal, the apparatus comprising comparator means arranged to discriminate the first signal, characterised in that the first signal represents a series of events and the normalisation apparatus further comprises means for spreading the first signal over a predetermined energy distribution, and means for automatically adjusting a discrimination level of the comparator means in response to a property of the second signal such that a proportion of the distributed signal is discarded by the comparator means.

The invention provides real-time normalisation of the first signal in a continuous and seamless manner without affecting properties of the first signal.

The energy distribution may be provided by a substantially random variation in the time domain of the amplification of the first signal. The energy distribution may for example be a voltage magnitude distribution or an electrical charge magnitude distribution.

Each of the events comprising the series of events represented by the first signal may be discarded or retained by the comparator means as a consequence of the normalisation process. The second signal should have a range of possible values, so that the discrimination level of the comparator means may be varied over a range of values. The second signal may therefore be an analogue signal or a multi-bit digital signal.

The invention is advantageous because it provides normalisation of a signal without requiring a mathematical divide function to be used.

Preferably, the first signal is representative of photons emitted or scattered by a sample in response to an excitation, and the second signal is representative of a property of the excitation. Alternatively, the sample may emit or scatter some other form of quanta, for example electrons.

Preferably, the predetermined distribution is a function of a detector arranged to detected the emitted or scattered photons. The detector may be a photo-multiplier tube. The detector may be an avalanche photodiode, super cooled PIN diode, or any other suitable detector.

The predetermined distribution may be provided by a dedicated electrical circuit. The circuit may provide a Gaussian distribution or other suitable distribution.

Preferably, the detected emitted or scattered signal is delayed in order that the normalisation may be synchronised with the excitation which gave rise to that signal.

The delay may occur after detection of the emitted signal, the delay being provided by an electrical delay line. Preferably, however the delay occurs prior to detection of the emitted signal, and is provided by optical delay of the emitted signal. The optical delay is preferred because it will not distort the emitted signal, whereas the electrical delay may introduce an element of distortion.

Preferably, the optical delay is provided by an optical fibre. The optical delay may be provided by an etalon. Other suitable forms of optical delay may be used, for example a scintillation optical fibre or a sample of a fluorophore.

Preferably the delay provided by the optical delay is variable. Coarse variability may be provided by means of switching between different lengths of optical fibre, whilst fine variability may be introduced by the use of an etalon, the delay being set by variation of gas pressure, temperature, mirror spacing, or number of reflections. The environment of the optical fibre may also be modified in order to vary the delay.

Preferably, the discrimination level of the comparator means is a function of the intensity of the excitation.

The discrimination level of the comparator means may vary according to the inverse of the intensity of the excitation. A linear variation is useful when scattering

measurements are being made. A nonlinear variation, for example a logarithmic variation, is useful when measuring fluorescence.

Preferably, a second comparator means is used to determine whether the intensity of the excitation falls within a predetermined range.

Preferably, the excitation is pulsed, and the discrimination level of the comparator means is determined by a first excitation pulse and then held at that level until a subsequent excitation pulse occurs.

Suitably, the discrimination level of the comparator means is a function of the energy of the excitation pulse. The normalisation may be carried out according a pulse width distribution instead of pulse energy or intensity.

Preferably, an output from the comparator means is directed to a plurality of counters, each counter being arranged to increment in response to signal pulses detected at a predetermined delay following an excitation pulse. The counters may be arranged to provide a pulse arrival distribution which varies logarithmically with respect to time. A counter may be arranged to increment once in response to a predetermined number of signal pulses, in order to avoid the occurrence of large numbers at that counter.

The discrimination level of the comparator means is preferably an upper level, such that that proportion of the distributed signal which lies above the discrimination level is discarded by the comparator means. The use of the upper level is preferred over a lower level because the lower level may introduce distortion of the signal due to thermal noise.

Alternatively, the discrimination level of the comparator means may be a lower level, such that that proportion of the distributed signal which lies below the value of the discrimination level is discarded. Preferably, the discrimination level is prevented from having a value which is less than a predetermined minimum.

Suitably, the adjustment of the discrimination level is achieved by adding or subtracting values from the distributed signal such that the predetermined energy distribution is shifted in relation to the discrimination level.

A variable delay may be introduced into the adjustment of the discrimination level of the comparator, to provide a phase difference measurement. The term phase difference measurement is intended to include a correlation of the detected signal with the excitation. The apparatus will provide a correlation regardless of whether the excitation signal is represented as an analogue signal or a multi-bit digital signal. The variable delay may be selected to measure a specific lifetime. Alternatively, the variable delay may be scanned over a range of values.

The normalisation apparatus may be provided with a series of comparator means, the comparator means being provided with different delays, thereby providing a phase difference measurement. Where this is done, the variable delay is not required.

At least one of the delays may be adjustable. This will allow a user to select a phase difference measurement at a particular delay or delays.

The time resolution of at least one comparator means may be adjustable, thereby providing an adjustable channel width for the phase difference measurement. The adjustment may be provided by a capacitor connected across the comparator means. A set of capacitors may be provided for connection across the comparator means to provide a desired channel width.

The time resolution of each of the comparator means may be adjusted by changing the integration time of at least one component which processes the second signal. This may be done by providing a capacitor across an amplifier which amplifies the second signal. Where this is done, the time resolution of each of the comparator means will change together.

At least one of the adjustments may be automated to locate specific features of the phase difference measurement. This may be done by using a set of rules, for example that the delay applied to a given comparator means be increased until the measured signal at a particular delay is less than the measured signal at longer and shorter delays, or until a gradient maximum is found, together with a rule that no two delays may be the same.

A variable delay may be applied to the first signal, thereby providing a further phase difference measurement. This is in effect a 'negative' delay, and may be useful when correlating a signal with itself, or cross-correlating periodically repeating signals.

The phase difference measurement may be used in the field of telecommunications, and in particular may be used to provide encryption. Where this is done, the second signal acts as a key and is convoluted with a digital signal on transmission. The digital signal is then deconvoluted by correlation using an embodiment of the invention which provides phase difference measurements.

The phase difference measurement may be used to allow rapid screening of a number of samples serially. The samples may include novel chemicals, drugs, materials, bacteria, viruses, prions, DNA, RNA or other biological components.

The comparator means may be a dedicated comparator circuit, or may be some other electronic component or components arranged to act as a comparator. For example, a transistor, or an electronic component known to act as a switch when operating near to its minimum or maximum voltage and/or current limit, with a transmission probability which may be affected by slight changes in control signal.

The excitation may be provided by a light source. Other suitable excitation sources may be used for example, sources of neutrons, electrons, magnetic field, electrical field, biological stimulus or chemical stimulus.

The mean value of the comparator means maybe measured. The lowest and highest settings of the comparator means may be measured. This allows analysis of the stability of the excitation source. This may be done for example during a light scattering experiment to measure the stability of the light source.

A low-pass filter may be used to remove interference or aliasing effects due to short time-scale cyclic fluctuations in of the excitation source

According to a second aspect of the invention there is provided a method of normalising a first signal representative of a series of events, with respect to a second related signal, characterised in that the first signal is spread over a predetermined distribution, and a discrimination level of a comparator means is automatically adjusted in response to a property of the second signal such that a proportion of the distributed first signal is discarded by the comparator means.

Preferably, a series of delays are introduced into the adjustment of the discrimination level of the comparator means, to provide a phase difference measurement.

Preferably, the delays are adjusted by a user in order to provide phase difference measurements at delays of particular interest.

The method may incorporate any of the above mentioned apparatus.

Specific embodiments of the invention will now be described by way of example only with reference to the accompanying drawings, in which:

Figure 1 is a schematic illustration of a normalisation apparatus according to the invention;

Figure 2 is a graph representing operation of the invention;

Figure 3 is a circuit diagram of part of the apparatus according to the invention;

Figure 4 is a circuit diagram of part of the apparatus according to the invention;

Figure 5 is a schematic illustration of a normalisation apparatus according to the invention;

Figure 6 is a schematic illustration of a normalisation apparatus according to the invention;

Figure 7 is a schematic illustration of a normalisation apparatus according to the invention; and

Figure 8 is a graph illustrating operation of the invention.

A specific embodiment of the invention is illustrated in Figure 1. A laser 1 generates a laser beam 2 which is directed at a beam splitter 3. A fraction of the laser beam 2 is reflected by the beam splitter 3 and is incident upon an optical detector 4 (hereafter referred to as the excitation detector 4). The remainder of the laser beam 2 is incident upon a suspension of particles 5, thereby illuminating the suspension 5. The laser beam 2 is scattered by particles in the suspension 5, and scattered light is coupled into an optical delay line 6, which in this case is a fibre-optic cable. Light carried by the fibre-optic cable 6 is detected by a photo-multiplier tube 7. The photo multiplier tube 7 may include a pre-amplifier to amplify its output (not shown). The signal from the photo-multiplier tube 7 is directed to a comparator 8 (hereinafter referred to as the detection comparator 8) which selectively discards a proportion of the detected signal and directs the remainder to a memory (not shown). The operation of the detection comparator 8 is described below.

An output signal from the excitation detector 4 is coupled to a comparator 9 (hereinafter referred to as the excitation comparator 9). The signal may be amplified before it reaches the excitation comparator 9. The excitation comparator 9 is provided with upper and lower discrimination levels derived from external inputs 10. The detected excitation signal is compared to the discrimination levels, and if the excitation intensity is below the lower discrimination levels then a first warning light 11a is switched on. If the excitation intensity is greater than the upper discrimination level then a second warning light 11b is switched on. The output from the excitation comparator 9 is amplified by an amplifier 12, and is then connected to an input of the detection comparator 8.

The detection comparator 8 has upper and lower discrimination levels which are pre-set prior to beginning an experiment. The position of the upper and lower detection discrimination levels is shown schematically as dotted lines 17, 18 in Figure 2. Figure 2 is a graph illustrating the number of photons detected or probability of detecting photons verses the energy of those photons. The distribution of high-energy photons 14 is due to cosmic radiation and/or after pulsing of the photo-multiplier tube 7. The decaying curve 13 shown at low energy corresponds to thermal noise from the photo-multiplier tube. The central distribution 15 corresponds to laser beam 2 photons which have been scattered by the suspension 5 and detected by the photo-multiplier tube 7. The energy distribution of these detected scattered photons arises from the photo-multiplier tube 7, and does not represent the energy of the scattered photons themselves. In fact, the scattered photons have a very narrow energy distribution as is illustrated by the line 16 at the centre of the photon distribution 15. The distribution of gain at the photo-multiplier tube 7 is primarily due to the statistical nature of the amplification at each dynode of the photo-multiplier tube 7.

In addition to the gain distribution that arises from the amplification at each dynode of the photo-multiplier tube 7, there may be a second effect which gives rise to a further energy distribution, namely the positions at which photons are incident upon the photo-multiplier tube 7. This effect is dependent upon the path of photons scattered by the suspension 5, and may add an unwanted distortion to the energy distribution. The effect is pronounced when a photon is incident upon the photo-multiplier tube 7 close to the edge of its effective area. In order to eliminate or minimise the effect, the edge of the effective area of the photo-multiplier tube 7 is masked (not shown). The effect is substantially eliminated by masking the outer 20% of the effective area of the photo-multiplier tube 7. Distortion of the energy distribution may be avoided by coupling photons scattered by the suspension 5 via an optical fibre (not shown) to the photo-multiplier tube 7. Where this is done, variation of the point of impact of the photons will not arise from properties of the scattering, but will instead be random.

The lower discrimination level 17 is set such that the majority of the thermal noise curve 13 is not detected, but little or none of the signal distribution 15 is lost.

Similarly, the upper discrimination level 18 is set such that photons due to after pulsing or cosmic radiation are not detected, but the little of the signal distribution 15 is lost. As mentioned above, the discrimination levels 17, 18 are pre-set before an experiment is begun. The setting of these two levels 17, 18 is known in the art.

The invention provides a third discrimination level 19 the position of which is determined by the detected excitation signal. This extra discrimination level 19 replaces the upper discrimination level 18, and any detected photons having energy above this level 19 are discarded by the comparator. The location of the discrimination level 19 varies as the intensity of the excitation laser beam 2 changes. If the intensity of the excitation laser beam 2 increases, the discrimination level 19 moves to a lower energy, thereby increasing the probability that a detected scattered photon will be discarded by the comparator. Similarly, if the intensity of the excitation laser beam 2 decreases then the discrimination level 19 moves to a higher energy, thereby decreasing the probability that a detected scattered photon will be discarded by the comparator. In this manner, the probability that a detected scattered photon will be directed to the memory is determined directly by the intensity of the excitation laser beam 2 from which that photon was derived. This is normalisation of the detected scattered photons in real-time. The discrimination level 19 does not introduce any distortion into the detected signal because each detected photon is randomly scattered within the distribution 15 (the distribution 15 is symmetric).

The invention effectively provides normalisation of each detected scattered photon, which may be part of a photon stream, according to the intensity of the excitation field which provided that photon. Furthermore, the normalisation may be carried out continuously and seamlessly.

The variable discrimination level 19 is adjusted in response to changes of the intensity of the excitation laser beam 2. The adjustment may proportional to the inverse of the intensity or may be some other function. The function may be nonlinear.

Although the invention is described in relation to a variable upper discrimination level 19, it will be understood that the invention could be similarly implemented with a variable lower discrimination level. Where this is done the lower discrimination level should be prevented from falling below a predetermined minimum value, to avoid introducing excess thermal noise into the normalised signal. A circuit configured to provide such a minimum value is shown in Figure 3. The minimum value is provided by the voltage source labelled V_{ref} which is connected to a 1 kΩ resistor. The input signal is provided at the point labelled INPUT. The circuit comprises a conventional arrangement of two cascaded inverting operational amplifiers. The first stage amplifier adds the input signal to the minimum value. The second stage amplifier is used to invert the summed signal to its original sign.

In general, the use of an upper discrimination level 19 is preferred because it avoids variation of the proportion of the thermal noise curve 13 included in the detected signal (the thermal noise curve often overlaps with the signal distribution 15, and is generally more significant than the cosmic radiation or after pulsing distribution 14).

In a further alternative arrangement, signal values may themselves be varied in order to obtain variable discrimination from a fixed comparator level. This may be conceptually visualised, with reference to figure 2, as moving the entire distribution 15 to a greater or lesser energy without altering its shape or its width (in effect adding or subtracting a given energy from every value in the distribution). Where a value is added to the distribution, the effect of the upper discrimination level 18 will be dependent upon the position of the distribution 15. A detected signal may be normalised by appropriate addition of values to an input signal in response to changes of the intensity of the excitation laser beam 2. This arrangement may be considered to provide an adjustment of the upper discrimination level 18, since it modifies the proportion of the distribution 15 which is cut off by the discrimination level 18.

Movement of the distribution 15 may be achieved by varying the bias of a transistor, for example using a circuit as shown in figure 4. Referring to figure 4, an input signal to be normalised is input to the base of a transistor, the bias of which is controlled by

a variable resistor A. The value of the resistor is altered in response to the intensity of the excitation laser beam 2.

Although the invention has been illustrated in relation to a photo-multiplier tube 7, the invention may also be implemented in any apparatus where a detected signal is broadened by detection apparatus to such an extent that the spread of energies of the photons incident at the detector may be considered to be a secondary effect. For example, in detectors where an electron avalanche is produced (e.g. avalanche photodiodes) a detection signal will have a broad distribution as a consequence of uncertainty in the gain of the detector. The gain uncertainty arises from variation of the location at which a photon is incident at the surface of the detector (this is effectively random due to the very small area of the detector), the depth within the detector at which absorption takes place, and the intrinsic bandwidth of an amplifier used to amplify the detected signal.

Where a solid state detector is used, the lower discrimination level 17 may be used to quench the detector following detection of a photon. This is necessary when the detector is of a type that may oscillate when a photon is detected, for example an avalanche photodiode. When the detector registers an event having energy greater than the lower discrimination level 17, a reset circuit is activated which resets the detector, thereby preventing oscillation.

Where the detected signal is not broadened sufficiently by detection apparatus, a distribution may be applied to the detected signal by a circuit provided specifically for that purpose. The circuit may be arranged to provide a Gaussian distribution or other suitable distribution.

The optical delay 6 is used to synchronise the detection comparator 8 to the excitation beam 2. The optical delay 6 may be constructed in any suitable form, for example an optical fibre as described above, or an etalon. A limited of amount of attenuation of the intensity of the scattered light may occur in the optical delay 6, but this will not influence the energies of those photons which are detected. The optical delay 6 may

be a monomode optical fibre, which has the advantage of restricting the coupling of light scattered by the suspension 5 to a single coherence area. Where the measurement of polarisation is important, a birefringent monomode optical fibre may be used as the optical delay 6 to preserve the polarisation information. A polarisation preserving fibre may also be used to act as a filter where one polarisation state is not required.

The optical delay may be provided by a fluorophore, the delay arising from time elapsed between excitation of the fluorophore and emission of a photon. The fluorophore may be in a bulk liquid sample. The delay provided by the fluorophore may be varied by altering its environment.

The fluorophore may be provided within a waveguide, for example a scintillation fibre. Scintillation fibres are known in the art and comprise short pieces of optical fibre which are doped such that they absorb incident photons and then emit photons following a fixed delay. The delay provided by the scintillation fibre may be adjusted using known adjustment methods.

Preferably the delay provided by the optical delay 6 is variable. Coarse variability may be provided by means of switching between different lengths of optical fibre, whilst fine variability may be introduced by the use of an etalon, the delay being set by variation of gas pressure, temperature, mirror spacing, or number of reflections. The environment of the optical fibre may also be modified in order to vary the delay, although this may introduce non-ideal optical fibre behaviour.

An optical delay 6 will not be required if the components which determine the value of the upper discrimination level 19 are sufficiently fast compared to the rate at which data may be output from the detection comparator 8. For example, if the maximum rate at which data may be output from the detection comparator 8 is 25ns, then no optical delay 6 will be required if the time elapsed between excitation light being incident upon the excitation detector and setting of the discrimination level 19 is equal to or less than 12.5ns.

The latched warning lights 11a, 11b allow a user to check if the excitation comparator 9 is operating within its intended range. When a warning light 11a,11b is illuminated the intensity of the excitation laser beam 2 may be modified in order to return the excitation comparator 9 to its operating parameters. The amplifier 12 may also be adjusted, although this may necessitate re-calibration of an experiment. A timing circuit (not shown) may be used to measure the period during which the excitation comparator 9 is operating outside of the intended range.

The above described embodiment of the invention may be used when a suspension 5 is illuminated continuously. Where the excitation is intermittent (or pulsed), the position of the discrimination level 19 may be set in response to an excitation pulse and held at that position until a subsequent excitation pulse occurs.

The detected quanta may be normalised via the intensity of a detected excitation pulse or an integral of the energy of a detected excitation pulse. An embodiment of the invention which is arranged to provide normalisation for pulsed excitation is illustrated in Figure 5. The apparatus corresponds to that illustrated in Figure 1, except that the energy of the excitation pulse is integrated and passed to a sample and hold amplifier 20 prior to input to the detection comparator 8. The sample and hold amplifier 20 maintains a particular discrimination level 19 until a subsequent excitation pulse occurs, whereupon the discrimination level 19 is adjusted in accordance with the integral of that excitation pulse. The operation of the sample and hold amplifier 20 is controlled via a feedback loop 21.

That proportion of the detected signal which is not discarded by the detection comparator 8 may be recorded directly in a memory. Alternatively, the apparatus may further comprise means for processing the detected signal following discrimination, for example to provide a pulse arrival distribution. The apparatus shown in Figure 6 corresponds to that shown in Figure 5, but includes a counter 22 connected to an output of the detection comparator 8 which counts the number of photons detected following an excitation pulse. A second detection comparator 23 is

enabled a pre-determined time after the excitation pulse, and provides an output to a second counter 24. The second detection comparator 23 is prevented from incrementing before the pre-determined time has elapsed by a delay circuit 25. Further comparators with associated delay circuits and counters may be provided (not shown) in order to obtain a pulse arrival distribution representing photons detected from a sample as a consequence of an excitation pulse. The contents of the counters 22, 24 are transferred to a memory (not shown).

The first counter 22 will increment every time a pulse is output from the detection comparator 8. The counter 22 may therefore be required to increment to a large number of times. In order to avoid this possibility a circuit may be interposed between the comparator 8 and the counter 22, the circuit being arranged to output a single pulse each time it has received a predetermined number of pulses (for example eight pulses). This will decrease eight-fold the number of incrementations of the counter 22.

The counters 22, 24, etc. may be arranged to provide a pulse arrival distribution which varies logarithmically with respect to time by suitable selection of the delay circuits 25, etc. This is advantageous when measuring for example an exponentially decaying fluorescence lifetime.

The embodiment of the invention illustrated in Figure 6 may be used to detect a signal received in response to a series of excitations, or continuous excitation over a pre-determined period (the sample and hold amplifier 20 may be used or bypassed as appropriate).

Where the bandwidth of the apparatus permits, logic circuits (not shown) may be incorporated into the embodiment shown in Figure 6 such that the first counter 22 will measure only events prior to the first delay, the second counter 24 will measure events between the first and second delays, and so on. Alternatively, the same effect may be realised by subtracting from the counters 22, 24, etc. the contents of all counters governed by longer delays.

The normalisation may be carried out according a pulse width distribution of the excitation instead of pulse energy or intensity.

The invention may be used to provide phase difference measurements (i.e. cross correlation of emission and excitation). This may be done by locating a variable optical delay (not shown) before the excitation detector 4. When the value of the variable optical delay is zero, the upper discrimination level 19 of the detection comparator 8 is synchronised to the excitation beam 2, and the normalisation of the detected experimental signal is in phase with the excitation beam 2. The experimental signal is measured for this zero delay. The variable optical delay is then incremented to introduce a slight delay in the setting of the upper discrimination level 19 of the detection comparator 8, so that the normalisation of the experimental signal is in relation to an excitation which occurred just before the emission. Again, the experimental signal is measured. The variable optical delay is incremented several times, and the experimental signal measured each time. This provides a correlation of the experimental signal in relation to the excitation signal. The magnitude and number of the incrementations may be selected by the user of the apparatus. This embodiment of the invention requires that the excitation signal is regular rather than random, so that the experimental signal at a given delay following excitation does not vary over time.

Phase difference measurements may be carried out without using a variable optical delay located before the excitation detector 4. A phase measurement apparatus is illustrated in Figure 7. The apparatus includes a series of detection comparators 8a, 8b, 8c governed by a series of delay circuits 25, 26 (although only three detection comparators are shown, together with associated delay circuits, the invention may utilise any number of comparators and delay circuits). The upper discrimination level 19 of the first detection comparator 8a is synchronised to the excitation beam 2, and the normalisation of the detected experimental signal (i.e. photons either emitted or scattered from a sample) is in phase with the excitation beam 2. The first delay circuit 25 delays movement of the upper discrimination level 19 of the second detection

comparator 8b, so that the normalisation of the experimental signal is in relation to an excitation which occurred just before the experimental signal was generated (i.e. before the photons were scattered or emitted from the sample). The second delay circuit 26 delays movement of the upper discrimination level 19 of the third detection comparator 8c, so that the normalisation of the experimental signal is in relation to an excitation which occurred a longer time before the experimental signal was generated. In each case, the normalised experimental signal is logged by counters 27a-c connected to the comparators 8a-c..

The signal from a given comparator, for example 8b, corresponds to the experimental signal normalised in relation to an excitation which occurred a predetermined time before the experimental signal was generated, the predetermined time being fixed by the delay circuit 25. However, the predetermined time will only be defined to an accuracy which is determined by the bandwidth of the comparator. In other words, the phase difference measurement will comprise a series of measurements at various delays, the delays having a time resolution determined by the bandwidths of the comparators. The time resolution is effectively the channel width of the measurements comprising the phase difference measurement. It may be desired to provide a phase difference measurement having low resolution (wide channels). This may be done by increasing the integration time of the comparators, for example by adding capacitors (not shown) to each of the comparators 8a, 8b, 8c, etc. so that their resolution is diminished (each comparator may include a set of capacitors or other suitable components which allow a user to obtain a preferred channel width). This allows the resolution of each comparator to be adjusted independently. Alternatively, or additionally, the integration time of the amplifier 12 may be adjusted. This will adjust the resolution at all of the comparators 8a, 8b, 8c, etc. simultaneously. The delay circuits 25, 26, etc. should be adjusted when the resolution is adjusted, to avoid overlapping channels.

This embodiment of the invention is particularly useful when measuring characteristic fluorescence lifetimes particularly when the characteristic lifetime includes more than one exponential component or a sample comprises more than one species of

fluorophore. Both the channel width and temporal location (i.e. the delay provided by the delay circuits 25, 26) of the measurements comprising the phase difference measurements may be modified by a user to allow the accurate determination of characteristic fluorescence lifetimes.

In the case of fluorescence analysis, a minima will be seen in the measured signal, which will correspond to the lifetime of the fluorescent sample being analysed. Where the sample contains different components each giving a different fluorescence lifetime, separate minima will be seen in the measured signal. A phase difference measurement of a fluorescent sample may be made in two stages. In an initial stage the delays and channel widths are fixed, and the approximate locations of minima in the detected signal are determined. In a second stage the delays and channel widths are adjusted by a user to provide high resolution measurement at the minima, thereby giving high resolution measurement of the locations of the minima.

In an alternative measurement arrangement, the delay applied to each comparator may be varied automatically by a processing unit in accordance with a set of rules such that the locations of minima are determined automatically. The rules could be for example that the delay applied to a given comparator be increased until the measured signal at a particular delay is less than the measured signal at longer and shorter delays, or until a gradient maximum is found, together with a rule that no two delays may be the same. The channel width may also be adjusted automatically, narrowing once a minima has been located in order to determine with high resolution the temporal location of that minima. This method may require that channel widths less than a certain cut-off value are ignored to ensure that the measurement is not over-resolved. This measurement arrangement allows the location and width of minima to be analysed in real-time. The measured output may be made to readout to a computer or other display device allowing a user to see the evolution of the measured signal toward specific correlator channel widths and temporal locations. The measurement may be made to stop after a given number of iterations, or once the minima have been located to within a certain value, or preferably when further iterations do not further

reduce the fitting error. The depth of a minima will be indicative of the quantity of the fluorophore associated with that minima.

Where the likely location of minima are already known, the apparatus may set up with the delays fixed at appropriate values. This may be of particular use in fluorescence screening, where the possible fluorophore lifetimes that may be present within a sample are known.

This embodiment of the invention allows phase difference measurements to be carried out that is dramatically different to a conventional correlation. Conventionally a number of channels, or delays, are set at a fixed spacing pattern, which is a multiple of the control clock pulses. Instead of this, the invention allows for the channels to have any delay. Furthermore, in a conventional measurement the channel width is fixed, whereas the invention allows for adjustment of the channel width. The invention thus allows the measurement of fluorescence lifetimes using less correlator channels than are required by the prior art.

Figure 8 illustrates the operation of the embodiment of the invention illustrated in Figure 7. Each correlation channel is indicated as a pair of lines 28a-e. The correlation channels are nonlinearly spaced, and have difference widths, in order to maximise the amount of information obtainable from the channels.

The excitation source 1 must be modulated, pulsed or varied in some way in order to carry out phase difference measurements (if there is no variation of the excitation source then there is effectively no phase information). The excitation source variation may be of a variety of types, including square, or sinusoidal wave excitation sources, trains of single pulses of a sine or square function and erratic CW sources. There is no requirement that the excitation source provide an excitation signal which is known or stable. Indeed, an irregular variation is preferred over for example a sinusoidal variation because this will include a range of frequency components.

In the above described phase difference measurements, the discrimination level is set following a delay so that the emission is normalised in relation to an earlier excitation. A further measurement may be obtained by including no delay when setting the discrimination level, and applying a range of delays to the detected experimental signal. This will provide a 'negative' delay. The 'negative' delay will only provide experimental information if the excitation is periodically repeated. A combination of measurements taken using positive and 'negative' delays may be made.

The 'negative' delay will also be useful when correlating a signal with itself, for example an excitation signal. In order to do this, the excitation signal must be represented in an analogue or multi-bit manner, and the detected experimental signal (in this case a small fraction of the excitation signal) must be represented as a series of discrete events (i.e. digitally). This measurement is in effect an autocorrelation, and may be less noisy than conventional analogue autocorrelations. An autocorrelation measurement of this type may be used to provide dynamic light scattering measurements.

The invention provides measurement of the phase difference between an analogue or multi-bit signal and a single bit digital signal (where the excitation signal is analogue or multi-bit and the detected experimental signal is a single bit digital signal). This measurement was not possible using prior art apparatus. The measurement is ideally suited to fluorescence phase measurements. The invention allows many emission pulses to be measured per peak of excitation, and thus provides rapid measurement of for example a fluorescence lifetime.

Similarly, the invention provides normalisation of a single bit digital signal in relation to an analogue or multi-bit signal. Again, this measurement was not possible using prior art apparatus. As described above, the normalisation measurement may be applied to the measurement of scattering from a suspension of particles, or may be applied to the detection of fluorescence. Indeed, the normalisation measurement may be applied to any pulse stream generated in response to an excitation.

The above embodiments of the invention have been described in terms of an excitation signal and light scattered or emitted in response to the excitation. However, it will be appreciated that the invention is not limited to these examples, and may be applied to any signal (analogue or digital) which is normalised in relation to an analogue (or multi-bit digital) signal. Examples of applications of the invention include fluorescence correlation spectroscopy, photon correlation spectroscopy, laser interferometry, telecommunications. In the field of telecommunications the invention may be used to provide encryption. Where this is done, the excitation signal (or an equivalent signal) acts as a key and is convoluted with a signal on transmission. The signal is then deconvoluted by correlation using an embodiment of the invention which provides phase difference measurements.

The phase difference measurement provided by the invention has a resolution which may be varied from close to DC to a few GHz. This is advantageous over the prior art, which requires analogue correlators to provide high speed (for example, GHz) measurements, and digital correlators to provide low speed measurements. Prior art analogue correlators cannot operate at low speeds because the accumulators which accumulate the signal begin to leak. Digital correlators cannot provide high speed measurements because the must multiply the excitation signal by the detected experimental signal, and this requires a shift register and a multiplier both of which are limited in their speed of operation.

The use of phase difference measurement to determine a fluorescent lifetime provides an advantageous level of signal to noise.

Where the invention requires two separate detectors 4,7, these may be produced on a single substrate or in the same housing. Detectors arranged to detect photons having different energies may be stacked on top of one another, thereby appearing as a single detector.

Detector and feedback circuits may be produced as a single intregrated circuit, which may include other components of the illustrated embodiments as appropriate.

The detectors 4,7 may be avalanche photodiodes, super cooled PIN diodes, or any other suitable detector. The output of the excitation detector 4 may be digitised.

The excitation field may be of many forms, for example photons, neutrons, electrons, magnetic field, electrical field, biological stimulus or chemical stimulus.

The comparator may be a dedicated comparator circuit, or may be some other electronic component or components arranged to act as a comparator. For example, a transistor having a control voltage which is close to its minimum turn-on value, will transmit pulses with a probability which may be varied by small changes of the control voltage, thereby acting as a comparator. Many other electronic components are known to act as switches when operating near their minimum or maximum voltage and/or current limits, with a transmission probability which may be affected by slight changes in control signal. These components may be used as comparators.

The total number of pulses that pass the lower level comparator 17 and the total number of pulses that pass the variable upper level comparator 19 may be counted, to assess the operation of the variable upper level comparator 19.

The mean value of the voltage that sets the comparator during a given experiment may be measured. A total count of pulses may be used to determine statistical properties.

In many applications it may be beneficial to measure the lowest and highest settings that occurred on the comparator, or the variance in the comparator setting value, during an experiment. This will allow analysis of the stability of the excitation source. This may be done for example during a light scattering experiment to measure the stability of the light source.

It may be preferable add a low-pass filter between the amplifier 12 and the detection comparator 8, to remove interference or aliasing effects due to short time-scale cyclic fluctuations in the excitation source

It may be beneficial to place an optically active sample immediately prior to the excitation and /or emission detectors to provide a signal filter. Many optically active materials, such as optically quenchable fluorophores, show non-linear behaviour allowing complex filters to be produced.

Where the optical delay 6 is provided by a fluorophore, the resolution of the normalisation apparatus may be affected by the inherent uncertainty in the fluorescent lifetime. This uncertainty may be used to modify the instrument bandwidth via selection of a suitable fluorophore.

Although use of the invention will reduce the total number of detected photons, it will often be possible to compensate for this by increasing the intensity of the excitation or the gain of the detector. Furthermore, statistically, a reduction of the number of detected photons of for example 10% will not significantly reduce the accuracy of a measurement of a characteristic decay time.